Forage Quality

Nutritive Value of Silage Corn Harvested at Two Heights Above Ground for Lactating Cows
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Introduction

The use of corn silage is increasing in dairy diets in some regions of the United States. Corn is increasingly being grown on land that is subject to soil erosion. Can we leave more of the lower stalk in the field to reduce soil erosion by providing surface cover? Also, can we improve the nutritive value of silage that is cut at a greater height to compensate for the loss of DM yield with the higher cutting height?

Materials and Methods

Forty-six Holstein cows (15 primiparous and 31 multiparous) averaging 112 days in milk were utilized in a crossover design experiment. The two treatments were corn silages harvested at two different heights (14" or 28" above the ground, respectively). The experiment included two periods, each lasting 4 wk. At the beginning of the trial, cows were divided into two groups based on similarity in parity, milk yield, and days in milk. The two groups were each assigned to a treatment during period 1. During the second period, cows received the opposite treatment. Cows were administered bST (Posilac; Monsanto Co., St. Louis, MO) every 2 wk beginning at the first week of the trial.

Each group of cows was housed in a free-stall barn and offered a TMR once daily ad libitum (5 to 10% refusal). Both diets contained 40% corn silage, either low or high cut. Actual amounts of feed offered and refused by each group were recorded daily to obtain net DM intake. However, only the last three weeks' records in each period were used for analysis; the first week was considered as a transition time.

Data on milk yield and milk composition were analyzed by the general linear model procedure of SAS (1985) using a model that included cow, period and treatment as the independent variables.

Results and Discussion

Cutting at 28" rather than 14" had a large effect on nutrient analyses of the silage (Table 1). Dry matter content of the ensiled material increased from 36.4 to 42.8%. The lower portion of the corn stalk is very low in DM content; about half as much as the average for the rest of the plant. Cutting at a greater height would have the practical benefit of yielding a drier silage. This could be advantageous in widening the window for harvesting, and/or in getting silage in a more desirable DM range for storage in tower silos. Quality of the silage was also markedly improved with higher chopping height. NDF and ADF were reduced by about 7 and 4 percentage points, respectively. Crude protein was not affected.

Table 2 shows the nutrient content of the total mixed diets for the two treatments. Nutrient content of the total mixed diets reflected the nutrient content of the treatment corn silages.

Dry matter intake, milk production and milk composition is shown in Table 3. Since the cows were group fed, statistical analyses on dry matter intake was not possible. It did appear that dry matter intake was slightly lower with the high cut silage. This might be expected if digestibility of the silage was increased.

Milk production was 1.2 kg per cow per day greater ($P < .01$) with the high cut silage, however 3.5% FCM was not different. Milk fat percent was reduced with the high cut silage, most likely reflecting the lower fiber content of the high cut forage. Milk protein was not different. If corn silage is cut at a greater height, it will be important to adjust the ration formulation to reflect the lower fiber content.

Accurate silage yield measurements were not made in this experiment. From other observations we have made, however, it would appear that leaving an additional 14" of stalk would reduce DM yield by 5-8%. Yield of wet silage would be reduced by about twice that amount because of the low DM content of the lower stalk. Loss of digestible nutrients were not measured in this study, but would probably be around 3-4%. These estimates apply to corn silage where the grain represents about 50% of total plant DM. Losses, expressed as a percent of plant DM, will be greater in situations where grain content is appreciably less.

Visual observation of chopped stubble indicates considerably more ground cover with the high cut silage. It remains to be seen how effective this can be in reducing soil erosion.

Conclusion

Cutting height can have a marked effect on moisture and fiber content of the resulting silage. Higher milk production was achieved with the high cut corn silage. Lower fat test was observed with the high cut silage, indicating the need to balance diets for the lower fiber content. Cutting at 28" can leave residue in amounts that may be effective, when chopped, in providing ground cover.

Table 1. Nutrient analyses of corn silage cut at two different heights.

Item	Low cut (14" above ground)	High cut (28" above ground)
	----------(%)----------	
DM	36.4	42.8
NDF	40.9	34.0
ADF	25.4	21.3
CP	8.4	8.3

Table 2. Nutrient content of diets containing corn silage cut at two different heights.

Item	Low cut (14" above ground)	High cut (28" above ground)
	----------(%)----------	
DM	53.9	56.4
CP	17.8	17.8
NDF	29.4	26.6
ADF	19.1	17.4

Table 3. Performance of cows fed diets containing corn silage cut at two different heights.

Item	Low cut (14" above ground)	High cut (28" above ground)	SEM	*P*
DMI, kg/d	20.8	19.8	...	...
Milk, kg/d	33.9	35.1	0.2	0.01
3.5% FCM, kg/d	35.0	34.5	0.4	0.42
Milk fat, %	3.74	3.39	0.06	0.01
Milk protein (true),%	3.11	3.12	0.02	0.44

Improving Use of Near Infrared Reflectance Spectroscopy Calibrations Among Laboratories of a Network

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Introduction

Forages represent about 50 % of the diet in lactating dairy cattle and larger percentages of other ruminant animals. Information about the chemical composition of forages is necessary to correctly balance nutrients in the diet. However, chemical and nutritional composition of forages is highly variable. Sources of variation include botanical composition, stage of maturity at harvest, harvest and storage method and climatic conditions. Near-infrared reflectance spectroscopy (NIRS), a rapid nonconsumptive method of analyzing forage crops was first identified by Karl Norris, USDA-ARS.

Many commercial forage testing laboratories use NIRS to analyze forage samples for dairymen, commercial hay producers and other livestock owners. Farmers are concerned about accuracy of forage test results and repeatability of sample analyses from lab to lab. Computerized transfer of NIRS calibration equations from one instrument to another, laboratory to laboratory, has been established as a satisfactory solution to reducing laboratory-to-laboratory variation. We propose a model NIRS network to support development of universal calibration, which can be distributed by a master instrument to slave instruments within the network.

Material and Methods

A network of commercial and public laboratories, NIRS Forage and Feed Testing Consortium, http://www.uwex.edu/ces/forage/NIRS/home-page.htm has been in operation since 1992. Experiments conducted with laboratories will be used to demonstrate to establish criteria of a model NIRS network to test forages for farmers in North America.

Laboratories have scanning monochrometer instruments, which collect reflectance information over 400 to 2500 nm, using NIRSystems instruments, models 4500, 5000, and 6500. NIRSytems WinISI software is utilized to run network calibrations. The network instrument operator utilizes ISI software for instrument diagnostics, instrument monitoring, and calibration monitoring. Each instruments spectra output is matched to a master instrument. A 30-cell characterization and 14-cell forage system are used to match instruments, which spectra trimmed to 1300-2500 nm, the region of the least expansive instrument. Routine samples are microwave dried and ground through cyclone grinders fitted with 1 mm screens. Samples are mixed before spectra and reference method analysis. National Forage Testing Association (NFTA), recommends reference methods utilized within the network. Network calibrations are developed using protocol established by National NIRS network. Calibrations are monitored using the network protocol.

Results and Discussion

Use of a network of NIRS instruments designed to use universal calibration equations has potential to deliver accurate forage test results among laboratories across North America. Such a network requires standardized drying and sampling processing, a method of monitoring accuracy of reference methods, harmonization of the instrument network, and calibration monitoring and development.

Standardization of drying and sample processing. Experience of operating our network has supported the recommendation that samples are dried alike, cyclone mills using 1 mm screens, and uniform sample processing, adequate mixing of samples before packing into cups for NIRS analysis as well as before reference method analysis. Large samples often dictates two separate grinds; grinding in a Willey mill using a 6 mm screen to break the large sample into smaller pieces rapidly followed by the cyclone mill. A segment of our network, alfalfa plant breeders have investigated elimination of the later grind using only one grind to a using a 2 mm screen to save time and labor. The network calibration monitoring statistics shows this is possible: %CP, 0.83 and 0.77; % ADF, 1.66 and 0.85; % NDF, 1.76 and 0.85; and % IVDDM, 1.60 and 0.78 for SED © and R^2, for 1mm vs. 2 mm grind, respectively.

Accurate results are dependent on drying method for various parameters. The accuracy of ruminal degradable protein (RUP) of legume and grass silages is dependent on method of drying. When comparing silage samples freeze-dried to those either oven-dried or microwave-dried, Hoffman and associates found oven-dried samples were similar to freeze-dried, but microwave samples were different. A repeatability file was prepared using hay and silage samples split, dried with microwave and oven-dried. Spectra are collected of each drying method samples to prepare a repeatability file, a file, which allows spectra adjustment for drying treatment. The adjusted calibration equations compared to original calibrations for RUP and dNDF are shown in Table 1. Improvements can be made with the repeatability file; however, drying methods must remain consistent with the repeatability treatment to remain effective.

Accuracy of reference methods. Reference method accuracy must be as good as possible for NIRS calibrations to be accurate. A method of maintaining accurate reference methods is for network laboratories to participate in National Forage Testing Association proficiency program. Using spectra from samples, which have been tested, by laboratories receiving proficiency grade is a must.

Harmonization of the instrument network. Software is available to match spectra output from scanning NIRS instruments. Ten years experience matching instrument spectra output to a master instrument for forage crop calibrations using a 30-cell characterization set and 14-cell forage set is available. The standardization set must be scanned by the master instrument, scanned by the slave instrument and rescanned by the master. For several years our network relied on successful standardizations using a 30-cell set. However, a test of the set determined the 95 % of check cells were leaking. Testing the original standardization made from the original standardization set collected at 7 laboratories against standardizations developed with a new forage set showed marked improvement in agreement among instruments, Table 2. (Standard deviation between 7 labs was improved by as much as 72 percent for % NDF).

Monitoring instrument performance via diagnostics is a must. Instrument manufactures recommend daily diagnostics with a check cell test being saved weekly. Our recent experience using a web-based diagnostics-monitoring program has been very successful. The program shows instrument operators there instrument performance statistics and also alters the network operator of instrument failures.

Calibration monitoring and development.
The final, but essential component of NIRS network operation is monitoring calibration performance. Selection of samples tested by laboratories within the network, selection based on monitoring instrument spectra supplied to network operator, provides the network validation of performance of the calibration used by the network. The network hay calibration, n = 1013, was updated using 654 samples from the network and 10 NFTA proficiency samples. Validation statistics of 2 updated

calibrations and a new calibration developed using "local" a new calibration model developed to be used with large data sets by 4 laboratories is shown in Table 3. In most cases the local calibration improved the calibration performance.

Conclusions

The potential to produce small deviations between laboratories testing forage crops with NIRS has been demonstrated. Standardizing drying and processing methods, monitoring reference method accuracy, harmonizing standardized instrument performance via web based programs, and monitoring calibration equations are keys to providing accurate forage tests between laboratories.

Table 1. The effect of drying method on ruminal undegradable protein and digestible neutral detergent fiber calibration performance.

	Original		Adjusted		Original		Adjusted	
Lab	SED	Bias	SED	Bias	SED	Bias	SED	Bias
	-----RUP, % of CP------				--------dNDF, % of DM----------			
Hays								
1	6.40	5.40	2.20	1.02	3.76	-2.56	1.81	0.97
2	6.60	-6.25	1.75	-1.15	2.75	-2.22	1.26	0.04
3	4.63	-3.35	1.75	-0.69	2.81	-0.87	1.39	0.04
Haylages								
1	2.14	1.16	1.12	0.38	1.41	-0.15	1.14	0.15
2	2.58	-2.20	1.17	-0.91	1.12	0.27	1.15	0.25
3	2.14	0.11	1.65	-0.10	1.73	-0.55	1.46	-0.41

Table 2. Improvement of laboratory network instrument performance from replacing defective standardization sample set.[1]

	Old standardization set				New standardization set			
Lab	DM	CP	ADF	NDF	DM	CP	ADF	NDF
	----------% of DM----------							
1	90.74	21.48	32.09	37.84	90.97	20.84	32.13	38.89
2	91.07	21.08	32.32	38.94	90.97	20.84	32.13	38.89
3	91.63	21.26	31.95	39.01	91.63	21.28	32.06	38.69
4	91.35	21.40	31.54	38.62	91.50	21.07	31.97	38.61
5	90.72	21.35	31.76	39.22	90.80	21.05	32.03	38.84
6	91.61	21.34	31.98	38.14	90.90	21.12	31.83	38.78
7	91.73	21.26	31.09	38.14	91.64	21.20	32.27	38.85
Mean	91.26	21.31	31.82	38.62	91.20	21.06	32.06	38.79
SD	0.43	0.13	0.40	0.49	0.37	0.17	0.14	0.11

[1]Monitored over 6-month period.

Table 3. Calibration monitoring statistics from 3 different updates including evaluation of a new local concept, 'Local'.

Method	SEP	Bias	SEP–C	R^2
	----------% of dry weight----------			
Crude protein				
LH0801	.80	.17	.78	.90
LH1097	.90	.48	.80	.89
LOCAL	.69	-.02	.69	.92
Acid detergent fiber				
LH0801	1.83	-.36	1.79	.86
LH1097	1.98	-.58	1.89	.84
LOCAL	1.48	.09	1.48	.90
Neutral detergent fiber				
LH0801	2.05	-.27	2.03	.89
LH1097	1.97	-.54	1.89	.90
LOCAL	1.52	.09	1.52	.94

Improving the Nutritive Evaluation of Corn Silage: I. Variability in Chemical and Physical Characteristics and Their Interrelationships.

D. R. Mertens and G. F. Ferreira

Introduction

Evaluation of corn silage provides a unique challenge because it contains variable proportions of grain and vegetative matter each of which can differ in availability due to chemical composition and physical form. When animals consuming corn silage do not perform as expected based on fiber level, it is uncertain if the discrepancy is due to altered proportion of grain in the silage, energy availability of the grain or stalk, or a combination of factors. Although chemical composition ultimately determines the availability of energy at the cellular and molecular level, physical properties of forages can impact digestibility by altering access to tissues and surface area available for microbial colonization and fermentation. Compared to other forages corn silage is more susceptible to the impact of physical properties because corn kernels may be inadequately chewed by dairy cows and pass out of the digestive tract before digestion is completed.

Summative equations, which add the digestible amounts of neutral detergent fiber(NDF), protein, fat, starch and non-starch soluble matter, may improve the nutritive evaluation of corn silage. Some summative equations also alter nonfibrous carbohydrate or starch digestibility when kernel processors are used during chopping of corn silage. The (CPM) net protein and carbohydrate model uses digestion kinetics of various fractions of protein and carbohydrates to estimate the energy value of corn silage. However, little is known about the relationships among chemical composition and physical properties of corn silage that might impact feed evaluation systems. Furthermore, there is no quantitative system for estimating the extent of grain damage that occurs with different levels of chopping or kernel processing. The objective of this research was to determine interrelationships among chemical and physical characteristics of corn silage, including a physical method for assessing kernel damage.

Methods

Thirty-two corn silages were obtained from a commercial feed analysis laboratory based on diversity in DM, CP, ADF, NDF, starch and visual appraisal of particle size. For each characteristic, materials were selected to represent the mean of the population of all corn silages analyzed by the commercial laboratory and to represent materials that were plus or minus two standard deviations from the mean for each characteristic while keeping other characteristics close to their respective means. Ash, CP, ADF, and acid detergent lignin (ADL-72% sulfuric acid method) were determined by AOAC procedures. Dry matter was determined by oven drying at 55°C for 48h. The amylase-treated NDF (aNDF) was determined using both amylase and sodium sulfite. Starch was measured using a YSI Biochemistry analyzer after enzymatic hydrolysis (Dairyland Laboratories). Mean particle size was determined on dried samples using a vertical shaker and sieves with apertures of 19.00, 13.20, 9.50, 6.70, 4.75, 2.36 and 1.80 mm.

It was observed in a preliminary experiment that whole kernels and fragments >1/4 of a kernel were retained on sieves with apertures > 4.75 mm. Each silage (233 ± 89 g of wet material) was sieved undried for 15 min with a vertical shaker using sieves with apertures of 19.00, 13.00, 9.50, 6.70 and 4.75-mm, in addition to the pan. After sieving, kernels and kernel fragments on the sieves were manually collected and then dried (48 h at 55°C) and analyzed for starch content.

Results and Discussion

Average DM, aNDF, ADF, and ADL (table 1) of our corn silages were similar to the DM (35%), NDF (45%), ADF (28.1%) and lignin (2.6%) reported in the 2001 Dairy NRC. However, average CP was lower than that reported in the Dairy NRC (8.8%) and average ash was higher than NRC (4.3%). The standard deviation and range in minimum and maximum values indicate substantial variation occurs in the chemical and physical characteristics of corn silage and illustrates the importance of feed analysis in determining nutritive value.

Table 1. Chemical and physical characteristics of 32 corn silages.

	Mean	SD	Minimum	Maximum
DM, %	34.68	7.81	19.23	48.10
CP, %	7.80	1.36	5.68	12.49
Ash, %	5.07	1.52	3.09	9.62
Starch, %	25.23	5.68	12.23	36.17
NFC[a], %	40.92	6.93	27.36	56.60
aNDFom[b], %	43.01	6.12	29.36	54.12
aNDF, %	44.18	6.40	29.98	56.30
ADF, %	26.94	4.42	17.67	34.68
ADL, %	2.26	0.59	1.16	3.50
Starch, %	25.23	5.68	12.23	36.17
MPS[c], cm	4.18	1.40	2.05	7.26

[a] NFC = 100 - CP - aNDF - Ash - EE, where EE = 3.20.
[b] ash-corrected, amylase-treated neutral detergent fiber organic matter.
[c] Geometric mean particle size was determined with a vertical shaker.

Although R^2 were typically <0.30, there were significant relationships between corn silage DM (as an indicator of corn maturity) and aNDF, ADF, ADL, starch, grain, and ash. These relationships indicate that a corn silage containing 25% DM would contain 8.1% CP, 5.8% ash, 21.9% starch, 34.9% grain, 48.0% aNDF, 29.9% ADF, and 2.5% ADL. They also indicate that a corn silage containing 45% DM would contain 7.4% CP, 4.3% ash, 28.8% starch, 43.6% grain, 40.1% aNDF, 23.7% ADF, and 2.0%

ADL. Some of these values differ from those reported in the latest Dairy and Beef NRC. The decrease in ADL and NDF with increased maturity probably reflects the diluting effect of starch during grain filling

For this diverse set of corn silages good relationships were obtained between grain and silage DM, between ADF or ADL and aNDF, and between starch and NFC (Table 2). If grain DM is an important factor related to starch utilization, it appears that corn silage DM can be used to indicate grain DM and starch availability. The relationship between ADF and aNDF indicates that ADF is about 60% of the aNDF in corn silage, which is lower than for other forages. Although lignin increased with increased aNDF, the proportion of lignin in NDF did not increase with maturity as expected, in this diverse set of corn silages. (This may be related to the fiber characteristics of grain, which contains some aNDF but very little ADL.) The Beef NRC indicates that 100% of the NFC, which they call NSC, is starch. Our results indicate that NFC in corn silage contains a significant non-starch component as indicated by the negative intercept and slope less than 1.0. It appears that starch is less than 75% of the NFC in corn silage.

Table 2. Linear relationships between chemical characteristics (n = 32).

		Intercept (b_0)		Slope (b_1)			
Y	X	Coef.	P <	Coef.	P <	R^2	SE
Grain DM	CS DM	36.71	.01	0.561	.01	.770	2.43
ADF	aNDF	-1.01	.66	0.633	.01	.840	1.79
ADL	aNDF	-0.82	.10	0.070	.01	.575	0.39
CS Starch	NFC	-5.95	.02	0.762	.01	.865	2.12
CS Starch	aNDF	59.98	.01	-0.787	.01	.786	2.67

About 20% of corn silage DM consists of whole and large fragments of corn kernels (Table 3). If these kernels and fragments are inadequately chewed and digested by lactating dairy cows, they can represent a significant loss of energy value from corn silage. The proportion of grain in corn silage, which was calculated by dividing corn silage starch concentration by the concentration of starch in manually collected kernels and large fragments, represented about 40% of corn silage DM and varied from 20 to 50%.

Table 3. Proportions and characteristics of kernel and kernel fragments in 32 corn silages

	Mean	SD	Min	Max
Kernels and fragments[a], %CS DM	20.49	9.66	4.18	48.35
Starch, % grain DM	64.32	3.40	56.96	70.27
Grain DM[b], % CS DM	39.09	7.86	20.41	52.96
Starch >4.75[c], % CS DM	13.27	6.64	1.83	33.02
Starch >4.75[c], % CS starch	52.18	20.93	8.73	100.0

[a] Retained on sieves with >4.75-mm apertures (as a percentage of corn silage DM).
[b] Determined by dividing total starch in each corn silage by its grain starch concentration.
[c] Starch in kernels and kernel fragments retained on sieves with >4.75-mm apertures.

Utilization of starch can impact the utilization of energy in corn silage as evidenced by the improvement in performance when kernel processors are used. The proportion of total starch in corn silage that is in kernels or fragments retained on sieves with apertures >4.75 mm, averaged about 50% and

varied from 10 to 100%. It appears that the proportion of starch > 4.75 mm can be used as a quantitative estimate of kernel damage due to chopping and kernel processing (Figure 1). The upper left and lower right borders of the data in figure 1 indicate the extremes in kernel damage. Although information about chopping and processing of these silages was unavailable, it is logical to speculate that the upper left border represents corn silages that were chopped without additional processing. The line fitting these silages indicates that 100% of starch is in kernels and large fragments when silages are chopped to attain a geometric mean particle size of >4.5 cm. The line fitting the lower right border of the data in figure 1 represents the maximum extent of processing that can be attained when chopped to various geometric mean particle sizes. Data between these two borders represent variable processing effectiveness and indicate the proportion of starch in kernels and large fragments.

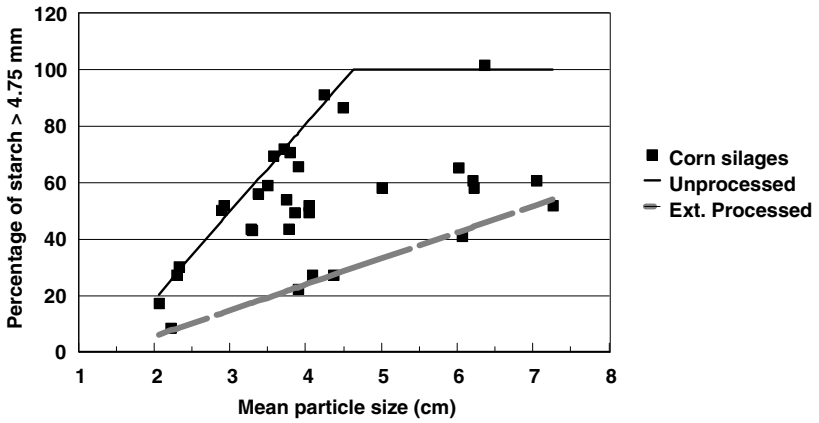

Fig. 1. Graph of the percentage of starch in kernels and large fragments (starch >4.75 mm) versus the geometric mean particle size of the silage with lines indicating unprocessed corn silage and extensively processed silages.

Summary and Conclusions

Average composition of a diverse set of corn silages agreed with values reported by the Dairy NRC. However, analyses of immature (25% DM) and mature (45% DM) differed from those reported in the Beef and Dairy NRC. Regression of starch versus NFC indicated that corn silage contains a significant non-starch fraction and that NFC is only about 75% starch in corn silage. Percentage of total starch in whole kernels and large fragments retained on sieves with apertures >4.75 mm provides a quantitative measure of kernel damage in corn silage, and may provide information useful in estimating processing adjustment factors for altering starch or NFC utilization when determining the energy value of corn silages.

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Improving the Nutritive Evaluation of Corn Silage: II. Identifying Factors Affecting In Vitro Fiber and Soluble Matter Digestibility.

D. R. Mertens and G. F. Ferreira

Introduction

In vitro "artificial rumen" methods are receiving renewed interest as approaches for improving forage evaluation. These methods were refined in the 1960's and made useful as research tools for comparing forages and understanding ruminal fermentation. The two-stage Tilley & Terry (T&T) method (or modifications of it) became the standard in vitro method for evaluating forages. This method involves a 48 h fermentation with ruminal inoculum followed by incubation in acid pepsin. In vitro dry matter digestibilities (IVDMD) of 1-mm ground materials measured by the T&T method correlate well with in vivo DM digestibilities measured at maintenance levels of intake. In the 1970's Van Soest replaced the second stage of the T&T method with neutral detergent (ND) extraction. Because ND solubilizes microbial debris, this method can be used to measure in vitro dry matter true digestibility (IVDMTD).

Evaluation of corn silage using in vitro methods is complex because a significant portion of its ND soluble matter (NDS) is starch. It appears that starch fermentation and digestion is affected by particle size. Grinding corn silage to 1 mm may result in IVDMD or IVDMTD that do not represent in vivo digestion, especially at production levels of intake when dairy cows may not chew corn kernels completely. A macro in situ method of measuring digestibility of corn silage has been proposed in which the fresh, whole silage is fermented with minimal physical disruption. This method probably underestimates in vivo digestion of corn silage because the effects of mastication are not mimicked.

The 2001 Dairy NRC indicated that a 48 hr in vitro fermentation could be used to estimate NDF digestibility when calculating TDN at 1X maintenance intake (TDN_{1X}). The TDN_{1X} is then reduced as a function of intake to estimate net energy value at production levels of intake. The objectives of this research were to assess the difference in IVDMD and IVDMTD of corn silages in various physical forms and to identify factors affecting the digestion of NDS and NDF fractions of DM.

Methods

The 32 corn silages selected to provide a diversity of chemical and physical characteristics were described previously. Dried silages were whole or ground through 4- or 1-mm screens and fermented in in situ (IS) bags. Sample amounts and bag dimensions varied to obtain about 9 mg/cm^2 of bag surface: Whole, 3.6 g in 10X20 cm IS (Wh-L) or 1.8 g in 10X10 cm IS (Wh-H); 4-mm, 0.90 g in 5X10 IS (4-mm); and 1-mm, 0.45 g in 5X5 cm IS (1-mm). Materials were fermented for 24 h in a rotating jar system using a media containing 400 mL strained ruminal fluid composited from 3 cows, 400 mL of buffer blended with ruminal solids from 3 cows, and 1200 mL of Van Soest media. After 24 h, pH and temperature of each jar were recorded and the bags removed. Bags were rinsed twice manually in ice water to stop fermentation, and once in a washing machine for 3 minutes. Twenty-four hours of fermentation was selected to minimize end product inhibition in the fermentation jars and to maximize differences among treatments. Washed bags were dried (24 h at 55 °C) and weighed to measure IVDMD without the second stage pepsin treatment.

In vitro residues from the Wh-L, Wh-H, and 4-mm materials were ground through a 1-mm screen before fiber analysis. All residues were extracted in neutral detergent using the filter bag system to determine true (IVDMTD) and in vitro aNDF digestibility (IVNDFD). Four replicates of digestibilities were measured in four in vitro runs with two incubators each containing four jars within each run. Samples were blocked by run, incubator, and jar location within incubator. Replicates of blanks and a corn silage standard were included in each jar within incubator within run. Digestibilities were corrected for blanks and means of replicates were used in statistical analyses.

Results and Discussion

The pH of media at the end of fermentation was not different among runs, incubators, or jar location within incubator (average pH=6.34). However, temperature of the media was different among days and jar location within incubator and did not agree with controller settings. Run 2 was cooler (37.6 °C) than runs 3 or 4 (38.1 °C), and we have no explanation for this difference. Jar location within incubator were numbered from 1 to 4, clockwise, starting in the upper left corner. Temperatures were 37.5, 37.9, 38.8, and 37.7 °C for locations 1 to 4, respectively. Location 3 was warmer and location 1 was cooler than other locations. This probably is due to the location of the heat source in the incubators and the direction of airflow.

Both IVDMD and IVDMTD of the corn silage standard differed between incubators (P>.09 and .04, respectively), but did not differ among runs or jar location within incubator. The pooled standard error for replication of corn silage standard IVDMD and IVDMTD were 1.73 and 1.57, respectively. Although there was no statistical difference in digestibilities among jar locations, there were high correlations (>.95) between jar temperature and IVDMD or IVDMTD. In vitro digestibility percentage increased 0.37 for each increase in degree centigrade.

A preliminary study indicated no difference in IVDMD between wet and dried silages and all digestibilities in this experiment were determined using dried samples. In vitro digestibilities of all components (DM, NDF, and NDS) were lower for whole material compared to 4- or 1-mm ground materials, but there was no difference in whole material digestibility when fermented in large or half-sized IS bags (table 1). The IVDMTD and IVNDFD of 4-mm ground materials were lower than that of the 1-mm ground silages, but IVDMD and IVNDS were not different between 4- and 1-mm grinds. The difference in IVNDSD between whole and ground silages, but lack of difference between 4- and 1-mm ground materials suggests that reducing particle size from whole to a 4-mm grind may destroy the physical effects of starch that inhibit its fermentation in vivo.

Table 1. In vitro[a] dry matter digestibility (IVDMD), true dry matter digestibility (IVDMTD), aNDF digestibility (IVNDFD) and neutral detergent solubles digestibility (IVNDSD) of 32 corn silages after 24 h of fermentation.

	IVDMD, %	IVDMTD, %	IVNDFD, %	IVNDSD, %
Whole-Large IS	61.1[b]	72.3[c]	37.4[c]	79.6[b]
Whole-Half IS	61.4[b]	71.7[c]	35.6[c]	81.2[b]
4-mm ground	70.8[a]	75.7[b]	44.9[b]	90.9[a]
1-mm ground	72.6[a]	77.4[a]	48.7[a]	91.2[a]

[a,b,c] Means with different superscripts within columns are different (P>.05).

Average correlation coefficients for IVDMD of 4- and 1-mm grinds were -.84, -.82, -.80 and +.75 for ADF, ADL, aNDF, and NFC, respectively. The correlations were similar for IVDMTD. Although the

summative equation approach includes a variable digestion coefficient for NDF, it implies that DM digestibility should be a function of NDF concentration, especially when physical limitations are minimized by grinding. The relationship between IVDMD and aNDF was improved when ADL was included in the equation:

IVDMD (1-mm) = 97.0 - .34(aNDF) – 4.14(ADL); R^2 = .68 and SEreg = 3.06.

Stepwise regression was used to determine which variables in addition to aNDF and ADL would explain variation in the IVDMD of whole and 4-mm ground materials. For whole material, dry matter concentration of corn grain (CG_DM) and starch >4.75 mm as a percentage of corn silage DM (StGT_DM) were the first additional variables included in regressions models:

IVDMD (Wh-L) = 136.6 - .54(aNDF) – 9.79(ADL) - .79(StGT_DM) - .34(CG_DM); R^2 = .67 and SEreg = 5.32 and

IVDMD (Wh-H) = 135.3 - .51(aNDF) – 7.02(ADL) - .42(StGT_DM) - .51(CG_DM); R^2 = .54 and SEreg = 5.49.

The regression coefficients for StGT_DM (an indicator of starch physical limitation) and CG_DM (an indicator of starch availability) indicate that these variables have a negative impact on digestion of whole silages. When these variables were include in the regression model for IVDMD of 1- and 4-mm ground materials they were not significant. This is additional evidence that grinding through a 4-mm screen eliminates the physical impact of starch on in vitro digestion. The corresponding equation for 4-mm ground material was:

IVDMD (4-mm) = 93.7 - .29(aNDF) – 4.44(ADL); R^2 = .82 and SEreg = 1.99.

The only variable that was consistently related to IVNDFD was the percentage of ADL in aNDF (ADL_NDF). Although regression coefficients were highly significant, lignin did not explain a large proportion of the variation in IVNDFD. The variables of mean particle size (a possible factor affecting whole material fiber digestion), corn silage DM (an indicator of maturity) or NFC (a potential depressor of fiber digestion) did not affect NDF digestibility:

IVNDFD (Wh-L) = 65.0 – 5.45(ADL_NDF), R^2 = .27 and SEreg = 8.05;
IVNDFD (Wh-H) = 61.2 – 5.05(ADL_NDF), R^2 = .16 and SEreg = 10.51;
IVNDFD (4-mm) = 60.5 – 3.08(ADL_NDF), R^2 = .24 and SEreg = 4.86; and
IVNDFD (1-mm) = 62.4 – 2.69(ADL_NDF), R^2 = .20 and SEreg = 4.84.

These equations suggest that the upper limit of 24 h IVNDFD for these corn silages was 60 to 65% and that lignin has a greater negative impact on the digestion of whole compared to ground material.

Regressions of digestible NDS versus NDS or NDS organic matter yielded high R^2 and regression coefficients (estimates of true digestibilities) near 1.00. Although the R^2 were low because the true digestibility of NDS is relatively constant, the proportion of total starch that was <4.75 mm (StGT_TS) had a significant negative impact on IVNDSD if whole materials:

IVNDSD (Wh-L) = 99.7 - .39(StGT_TS); R^2 = .49 and SEreg = 8.40, and
IVNDSD (Wh-H) = 90.6 - .18(StGT_TS); R^2 = .19 and SEreg = 8.00.

Summary and Conclusions

There were differences in temperature of the media at the end of fermentation among runs and locations within the incubator. Although in vitro digestibilies were not statistically different among locations within incubators, there was a significant relationship between temperature and IVDMD among the locations of jars. Whole corn silages have lower in vitro digestibilities compared to 4- or

1-mm ground materials. The IVDMTD and IVNDFD differed between and 4- and 1-mm ground materials. In vitro DM digestibility was related to aNDF and ADL concentration for all physical forms. The variables of dry matter concentration of corn grain and starch >4.75 mm as a percentage of corn silage DM improved the prediction of IVDMD for whole materials.

In vitro NDF digestibility was related to the percentage of ADL in aNDF in all physical forms. The true digestibility of NDS was near 100% for 4-mm and 1-mm ground materials. The proportion of total starch that was <4.75 mm had a negative influence on the IVNDSD of whole materials. It appears that particle size affects the digestibility of fiber and soluble matter in corn silages and this effect is nearly eliminated when silages are ground through a 4-mm screen. The negative impact of lignin on NDFD is largest in whole silages and the proportion of starch in whole kernels and fragments >4.75 mm affects NDSD.

Collaborative Study Demonstrates that the aNDF Method is Reproducible Among Laboratories

D.R. Mertens

Introduction

Analysis for NDF has the reputation for being more difficult and variable than determining ADF or CF. Differences in NDF methodology and poor laboratory technique are the most important and controllable sources of variation in NDF results among laboratories. Both problems can be minimized by following a standard NDF method exactly. Although the concept of fiber is based on nutritional criteria, the chemical measurement of fiber is defined by the laboratory method that is used. Modifications of the NDF method affect the "fiber" being measured, cause values to be different among laboratories, and give the mistaken impression that NDF cannot be measured accurately or precisely. Poor laboratory technique compounds these problems by increasing filtration difficulties and decreasing the effectiveness of washing fiber residues.

The original NDF method used a boiling detergent solution with sodium sulfite to remove protein and EDTA to chelate calcium and remove pectin. However, this procedure did not adequately remove starch from concentrates or silages that contained grains. The neutral detergent residue (**NDR**) method, which uses a heat and detergent-stable amylase to assist in the removal of starch, was developed to improve fiber determination in concentrates, but this modification of NDF eliminated the use of sodium sulfite because it might remove phenolic compounds thought to be lignin. Our laboratory developed a NDF method that can be used on all feeds and is both repeatable within laboratories and reproducible among laboratories. This method uses both amylase and sodium sulfite and is called the amylase-treated NDF (**aNDF**) method to distinguish it from other modifications of the NDF method. The objective of this study was to determine repeatability and reproducibility of the aNDF method for approval by the Association of Official Analytical Chemists International (AOAC) as an Official Method.

Methods

A detailed description of the method is too long to be included in a research summary. A copy can be obtained by contacting the author. In brief, the method includes sodium sulfite and uses two additions of heat-stable amylase to hydrolyze starch, one after bringing the reagents to boiling and one at the first residue-soaking step. The amylase solution is standardized to contain adequate activity in hot neutral detergent solutions. Fiber residue washing is described as a soaking procedure, blanks are included in each run, and residues may be ashed to obtain ash-free aNDF organic matter (aNDFom). Samples >10% fat are extracted with acetone prior to aNDF determination and modifications for difficult samples are described.

Twelve laboratories representing, research, feed company, regulatory and commercial feed testing laboratories analyzed 11 materials as blind duplicates. The materials represented a wide range of feed matrices including animal products, high protein feeds, high fat feeds, high pectin feeds, oil seeds, grains, heated byproduct feeds, and legume and grass hays and silages. Materials were selected to vary in chemical composition and contained 0 to 90% aNDF, 1 to 16% ash, 1 to 20% crude fat, 1 to 40% crude protein, and 0 to 50% starch.

Results and Discussion

Results of aNDF analyses were calculated four different ways (aNDF, aNDF blank-corrected, ash-free aNDF organic matter – aNDFom, and aNDFom blank-corrected) and the results for aNDFom blank-corrected are given in table 1. Outliers were detected using statistical tests recommended by AOAC. The laboratory ranking test indicated that Lab 12 was an outlier because it had an average bias of +1.99%-units of aNDF for all materials. Lab 12 indicated that they used medium porosity Gooch crucibles with ceramic fiber as a filter aid, which were not indicated in the aNDF method and their results were removed. Remaining outliers occurred mainly in two laboratories and in each case only one of the blind duplicates was suspect. This suggests that the aNDF method and its description may not be at fault because most remaining collaborators produced acceptable results and even the two laboratories with problems generated one acceptable result for all materials. The outlying results were eliminated from evaluation of the aNDF method.

Table 1. Statistical data for ash-free aNDF organic matter (aNDFom) that were blank-corrected.

Sample ID	n[a]	Mean (%)	s_r[b]	s_R[c]
Alfalfa silage	12	39.09	0.91	0.91
Brewer's grains	11	47.88	1.82	2.24
Citrus & beet pulp	12	27.36	0.75	1.08
Corn grain with cob	11	21.30	0.34	0.46
Corn silage	12	36.29	0.60	0.82
Corn stalks	11	69.27	0.98	1.46
Dairy mixed feed	12	12.09	0.67	0.89
Grass hay	11	55.83	1.16	1.38
Milk replacer	11	0.11	0.21	0.37
Roasted soybeans	11	13.38	0.59	1.63
Sawdust	10	89.01	1.74	1.74

[a] Number of laboratories.
[b] Standard deviation of repeatability within laboratories.
[c] Standard deviation of reproducibility within and among laboratories.

Blanks may account for systematic weighing variation among runs. The 95% confidence interval of blank variation by collaborating laboratories was about 10 mg. Because the weights of fiber residues are small, the effect of adjusting for weighing variation using blanks should be greater for materials with low aNDF. If a material contains 10% aNDF, the fiber residue from a .5 g test sample would weigh only 50 mg and the variation attributed to blank-correction could be 20%. When aNDF was blank-corrected, the average reproducibility standard deviation for materials containing <25% aNDF decreased from 1.30 to 1.01. Thus, blank-correction increases analytical precision for materials with <25% aNDF.

Analytically, ashing fiber residues and expressing results as aNDFom improves the reproducibility of results for materials with <50% aNDF. However, it decreases analytical reproducibility slightly for materials with >50% aNDF. Ashing fiber residues requires an additional step in the procedure, which incurs extra time and expense. For routine fiber analysis of forages, measuring aNDFom may not be cost effective, but there are nutritional benefits for measuring aNDFom. When nonfibrous carbohydrates (NFC) or neutral detergent soluble carbohydrates (NDSC) are calculated as dry matter - crude protein - crude fat - ash – aNDF, the ash included in aNDF is subtracted twice. Using aNDFom to calculate NFC would correct this error.

The average reproducibility standard deviation for all materials was 1.29, 1.24, 1.20, and 1.18 for aNDF, aNDF (blank-corrected), aNDFom, and aNDFom (blank-corrected), respectively. Although adjusting results for both ash and blanks improves precision, the small differences among results do not indicate a strong preference for one method of expressing the results over another. Thus, it is suggested that the method for expressing the data be left to the discretion of the laboratory and calculations for all four results are given in the aNDF method. It will be incumbent on the laboratory to state clearly which method for calculating results was used. The exception to this general rule of allowing analysts the option for choosing the method of expressing aNDF results is for feeds containing <25% aNDF. The average reproducibility standard deviation for these materials is 1.30, 1.01, 0.90 and 0.84 for aNDF, aNDF (blank-corrected), aNDFom, and aNDFom (blank-corrected), respectively. There is a clear advantage to ash- and blank-correction when materials contain <25% aNDF and this is the recommended method for these feeds and for regulatory laboratories.

Table 2 provides expected performance parameters for the aNDF method determined on categories of feeds. The standard deviation of repeatability within laboratories (s_r) was 50 to 80% of the total reproducibility within and among laboratories. This suggests that much of the variation in aNDF results is related to differences among test subsamples that are selected for analysis. The coefficient of variation or relative standard deviations were small for forages and concentrates with > 30% aNDF. The $2.8*s_r$ provides an estimate of acceptable differences between replicate analyses within a laboratory. Replicates outside this value should be reanalyzed. When performing single analyses, 19 out of 20 results among laboratories should be within ±R from table 2 (approximate 95% confidence interval).

Table 2. Performance parameters for the aNDF and aNDFom blank-corrected method.

Feed	Fiber	Mean (%)	s_r[a]	s_R[b]	RSD_r[c] (%)	RSD_R[d] (%)	r[e]	R[f]
Forages	ANDF	52.2	0.84	1.08	1.61	2.08	2.36	3.03
Forages	aNDFom	50.1	0.93	1.16	1.85	2.32	2.60	3.26
Concentrates <10% fat	ANDF	33.2	1.25	1.57	3.76	4.72	3.50	4.38
Concentrates <10% fat	aNDFom	32.2	1.14	1.47	3.55	4.56	3.20	4.11
Concentrates >10% fat	ANDF	8.7	0.79	1.24	9.06	14.26	2.21	3.47
Concentrates >10% fat	aNDFom	8.5	0.53	1.10	6.25	12.94	1.49	3.09
All materials	ANDF	38.6	1.02	1.28	2.64	3.32	2.86	3.59
All materials	aNDFom	37.4	1.00	1.24	2.67	3.32	2.80	3.48

[a] Standard deviation of repeatability within laboratories.
[b] Standard deviation of reproducibility within and among laboratories.
[c] Relative standard deviation ($100*s_r$/mean) for repeatability within laboratories.
[d] Relative standard deviation ($100*s_R$/mean) for reproducibility within and among laboratories.
[e] $2.8*s_r$ = approximate 95% confidence interval.
[f] $2.8*s_R$ = approximate 95% confidence interval.

Conclusions

Fiber is an important constituent of animal feeds because it represents the portion of feeds that is bulky and difficult to digest. The amylase-treated NDF (aNDF) method was developed as an accurate and precise method of measuring total insoluble fiber in feeds. A collaborative study was conducted to evaluate the repeatability and reproducibility of the aNDF method over the full range of feeds. Twelve laboratories representing, research, feed company, regulatory and commercial feed testing laboratories analyzed 11 materials as blind duplicates. The materials represented feed matrices including animal products, high protein feeds, high fat feeds, high pectin feeds, oil seeds, grains, heated byproduct feeds, and legume and grass hays and silages. Correcting results for changes in blanks and reporting results as ash-free aNDF organic matter improved the repeatability and reproducibility of results when aNDF was <25%. The within laboratory repeatability standard deviation for percentage aNDFom in feeds varied from 0.21 to 1.82 and the standard deviation of reproducibility among and within laboratories varied from .37 to 2.24. Both are within limits accepted by the Association of Official Analytical Chemists International and currently aNDF is being considered for Official Method - First Action status.

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